Amdt #7

PATENTS
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Applicant:	Murphy *et al.*	Art Unit:	1633
Serial No.:	09/523,809	Examiner:	C. Stroup
Filing Date:	March 13, 2000		
Title:	**Bioengineered Tissue Constructs and Methods for Producing and Using Thereof**		

Commissioner for Patents
Washington, DC 20231

DECLARATION OF NANCY PARENTEAU UNDER 37 C.F.R. § 1.132

Hon. Commissioner for Patents:

I, Nancy Parenteau, declare as follows:

1. I obtained my M.S. in 1977 in Biology and my Ph.D. in 1985 in Anatomy and have spent more than fourteen years involved in tissue engineering. I have authored or co-authored several papers, invited reviews/chapters, and abstracts. I have given several invited talks in this field. I am a named inventor on three patents. My curriculum vitae, including a list of these publications and presentations, is enclosed herewith as Exhibit A.

2. Currently, I am Senior Vice President and Chief Scientific Officer at Organogenesis, Inc. in Canton, Massachusetts, which is the assignee of the above-identified application.

3. Organogenesis, Inc. constructed the cultured tissue constructs used in the following detailed characterization by culturing fibroblast cells in serum-free conditions as

described essentially in Example 15, using condition 2, and in one variation the media was supplemented with 2% fetal calf serum as described in paragraph five below.

4. A detailed characterization of the extracellular matrix components produced by cultured fibroblasts under both serum and serum-free conditions was performed. As part of this detailed characterization, the presence of a quarter-staggered 67 nm banding pattern and decorin was determined.

5. Neonatal human dermal fibroblasts (P7) were harvested at confluence and seeded at high density on a porous polycarbonate membrane in a transwell format and periodically fed for twenty-one days with serum-free media supplemented with growth factors, ascorbic acid, vitamins and nutrients. In one variation of the culture method the media was supplemented with 2% fetal calf serum. The cells continuously produced matrix during this time period to create a tissue with uniform thickness. Histological analysis of the constructs at day twenty-one demonstrated the presence of the fibroblasts in what appeared to be a dense collagenous matrix that we termed Human Dermal Matrix (serum and serum-free). Ultrastructural analysis of the Human Dermal Matrices was performed by transmission electron microscopy (TEM). At high magnification banded collagen fibrils exhibiting the quarter staggered 67 nm periodicity characteristic of fibrils in human skin were seen in the matrix surrounding the fibroblasts (See Fig. 1, attached hereto as Exhibit B). On information and belief, these results demonstrate the presence of a quarter-staggered 67 nm banding pattern in the extracellular matrix of a tissue construct produced in the absence of exogenous matrix components.

6. Partially purified proteoglycan extracts of serum and serum-free Human Dermal Matrices were prepared and assayed for decorin by an inhibition ELISA (biglycan was also assayed). Three samples of 3.14 cm^2 were cut out from the Human Dermal Matrix constructs and weighed. Each sample was chopped into small pieces and extracted in 4 ml of 7M urea / 0.05M Tris-HCL / 0.15M NaCl / 0.01M EDTA / 0.0005M PMSF / 0.02% sodium azide, pH 6.8 at 4° C for 24 h on a rotator. The supernatant was collected by centrifugation and the extraction repeated for 72 h. Combined supernatants were applied to a column containing 1 ml DEAE-Sephacel (Pharmacia) equilibrated in extraction buffer. Bound proteoglycans were eluted with extraction buffer containing 1M NaCl. Eluates were desalted and concentrated to 0.5 - 1 ml using centrifugal ultrafilters (Millipore Ultrafree-15, 10K NMWL) and lyophilized. ELISA for

decorin was accomplished as follows: Eight-well strips (Nunc Maxisorb) were coated with the protein core of purified bovine skin decorin in a 1:1 mixture of Voller's buffer and PBS, overnight at 4°C. Wells were washed with PBS/Tween and then filled with 1% bovine serum albumin in PBS/Tween to block non-specific binding. After 4 h at room temperature the wells were washed again with PBS/Tween. Lyophilized extracts containing unfractionated proteoglycans were dissolved in 0.1 ml of 0.1M Tris/acetate, pH 7.3. Suitable dilutions of these solutions were prepared (in triplicate) in the same buffer, mixed with an equal volume (50 ul) of the monoclonal antibody 6D6 (hybridoma cell culture supernatant, diluted 1:400) and left on the plates overnight at 4°C. Standard solutions of decorin protein core were prepared to cover the range of 0 - 1120 ng per well. After a series of washes, the second antibody (alkaline-phosphatase-conjugated goat anti-mouse IgG, Sigma) was applied and color developed using the substrate p-nitrophenyl phosphate. The results demonstrated that serum-free Human Dermal Matrix contains twice as much decorin as the serum-containing construct (See Table, attached hereto as Exhibit C). On information and belief, these results demonstrate the presence of decorin in the extracellular matrix of a tissue construct produced in the absence of exogenous matrix components.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 4/12/01

Nancy L. Parenteau

Nancy L. Parenteau, Ph.D.
5 Mainstone Road
Wayland, MA. 01778

Place of Birth: Manchester, New Hampshire

Work Experience:

Organogenesis, Inc. 1986-Present (Organogenesis Inc. is a biotechnology company involved in tissue engineering, biomaterials and cell therapy)

1995-present	Senior Vice President and Chief Scientific Officer
1994-1995	Vice President, Cell and Tissue Science
1990-1994	Director of Cell Biology Research
1988-1990	Co-Director of Research and Living Skin Equivalent Program Director
1986-1988	Group Leader, Cell Biology

Post-Graduate experience:

1982-1986 Post- Doctoral Fellow, Laboratory of Toxicology, Harvard School of Public Health

Education:

- B.A., Zoology, 1975, University of Vermont, Burlington, VT
- M.S., Biology, 1977, Rivier College, Nashua, NH
- Ph.D., Anatomy, 1985, Georgetown University, Washington, D.C.

Field of Study:

- Academic Training: Anatomical Sciences with specialization in Developmental Biology with additional technical training in lymphocyte hybridoma production and immunochemistry.
- Master's Thesis: "An Analysis of the Action of Thyroid Hormone on the Development of the Early Chick Embryo", Dr. Claire Bileau, Advisor.
- Doctoral Dissertation: "Antigen Distribution in Pancreatic Cells of the Chick Embryo and Adult Studied with Monoclonal Antibodies", Dr. Gerald Goeringer, Advisor.
- Postdoctoral Research: Characterization of specific markers of keratinocyte differentiation and the study of their regulation in vivo and in vitro.

Teaching Experience:

- Medical Gross Anatomy, Teaching Assistant, 1978;
- Medical Neurobiology, Teaching Assistant, 1979
- Medical Embryology, Lecture Presentation, 1980, 1981
- Medical Microscopic Anatomy, Laboratory Instructor, 1981
- Human Anatomy and Physiology, Course Instructor, George Washington Univ. Hospital
- Radiation Oncology Program, 1981
- Junior Laboratory Instructor, 1982

Scientific Management Experience:

Responsible for overseeing multidisciplinary groups involved in research and product development. Currently directly responsible for the oversight of company research in the areas of:

- Cell Biology Research
- Morphology
- Molecular Biology
- Cryobiology
- Biomedical Engineering
- Matrix Biology
- Cell Culture Research and Development
- Immunobiology
- Product Development
- Tissue Engineering
- Orthopedics
- Vascular Biology

Significant Achievements:

- While at Harvard developed a highly sensitive immunoassay for involucrin (a specific marker of keratinocyte differentiation) now marketed by Biomedical Technologies.
- Working with Robert Rice, was the first to describe the unusually rapid evolution of the involucrin protein in primates.
- Developed a new defined method for keratinocyte cultivation which has increased our fundamental understanding of epithelial cell regulation and has led to the development of defined cultures systems for additional cell types. The method enables experiments that were previously impossible, enables microcarrier culture of keratinocytes, and enables the development of epidermal cell sheets for grafting without the use of undefined components or feeder cells. Development of this system was also critical for the development of the living skin equivalent; the major program and first product of Organogenesis Inc. Patent filed 1989.
- One of the principal developers of the Living Skin Equivalent, the company's first product for in vitro and clinical use. It is a manufactured human skin product that contains both epidermis and dermis. The clinical product is the first cell therapy to have successfully completed a large-scale clinical trial toward FDA approval. It is expected to be one of the first, if not the first, regulated cell therapy to reach the marketplace. The in vitro product won an R&D 100 award in 1990, which recognized our work as a significant technological advance leading to a product.
- Led team of in house staff and an academic consultant who achieved successful cryopreservation of the skin equivalent, maintaining viabilities of approximately 90% or greater. This accomplishment represents the state of the art in cryopreservation technology.
- Through my work at Organogenesis, have become established as a recognized leader in the field of tissue engineering and cell therapy.

Professional Memberships:

- American Society for Cell Biology
- Sigma Xi, Scientific Research Society, Harvard-Radcliffe Chapter
- Wound Healing Society
- American Chemical Society

Board memberships:

- Scientific Advisory Board – Harvard Forsyth Craniofacial Research Center
- Board member – Pittsburgh Tissue Engineering Initiative
- Panel member - World Technology (WTEC)

Miscellaneous:

- Cited as one of three principal developers of TESTSKIN®, recipient of the "R&D 100 Award" given by Research and Development magazine recognizing the 100 most significant technological advances of 1990 resulting in a commercial product.
- Cited by Fortune magazine in the "On the Rise" segment of "Fortune People" Dec. 1990 recognizing people with promising careers below the age of 40.

Publications:

Invited Reviews/Chapters

- Epidermis Generated in Vitro: Applications and Practical Considerations. N. L. Parenteau, C. M. Nolte, P. Bilbo, M. Rosenberg, L. Wilkins, S. Watson, E. Johnson and E. Bell. , J. Cell. Biochem 1991; 45:245-251.
- Keratinocyte Transglutaminase: Differentiation Marker and Member of an Extended Family. R. H. Rice, M. Mehrpouyan, W. O'Callahan, N. L. Parenteau and A. L. Rubin, Epith. Cell Biol 1992;1:128-137.
- Skin Equivalents. N.L. Parenteau in: Keratinocyte Methods, Leigh, B. Lane, and F. Watt, Editors, Cambridge Univ. Press, London, 1994, pp. 45-54.
- In Vitro Skin Models as Tools for the Testing of Topical Formulations, Kriwet, K., and Parenteau, N. L. Cosmetics and Toiletries 1996;111:93-101.
- Approaches to Transplanting Engineered Cells. J. Hardin Young, J. Teumer, P. D. Kemp, N. L. Parenteau in: Tissues in Principles of Tissue Engineering, Robert Lanza, Robert Langer and William Chick, Editors, R. G. Landes Company, 1997.
- Wound Research, Parenteau, N.L., Sabolinski, M.L., Mulder, G., and Rovee, D. in Chronic Wound Care, 2nd Edition 1997, Edited by D. Krasner and D. Kane.
- Tissue Engineered Skin. J. Teumer, J. Hardin Young, N.L. Parenteau in Frontiers in Tissue Engineering, 1st Edition 1998, Edited by C.W. Patrick, Jr., A.G. Mikos and L.V. McIntire.
- Skin: The First Tissue-Engineered Products. N. L. Parenteau in Scientific American, April 1999, pp. 83-84.
- Cell Differentiation. N.L. Parenteau in Encyclopedia of Animal and Plant Cell Technology, John Wiley and Sons, 1999, pp. 365-378.
- Bioengineered Skin: Manufacturing, Safety and Quality Control, L. M. Wilkins, N. L. Parenteau in Wound Healing and the Skin. In press.

- Skin. N.L. Parenteau, J. Hardin Young, R.N. Ross. In Principles of Tissue Engineering, 2nd Edition, R. P. Lanza, R. Langer and J. Vacanti (Eds), Academic Press 2000, pp. 879-890.

- Approaches to Transplanting Engineered Cells and Tissue. J. Hardin Young, J. Teumer, R.N. Ross, N.L. Parenteau In Principles of Tissue Engineering, 2nd Edition, , pp. 281-291,

Papers

- Induction of Keratinocyte Type-I Transglutaminase in Epithelial Cells of the Rat. N. L. Parenteau, A. Pilato, and R. H. Rice, Differentiation 1986; 33(2):130-141.

- Primate Involucrins: Antigenic Relatedness and Detection of Multiple Forms. N. L. Parenteau, R. L. Eckert and R. H. Rice, Proc. Natl. Acad. Sci. USA 1987; 84(21):7571-7575.

- Coordination of Keratinocyte Programming in Human SCC-13 Squamous Carcinoma and Normal Epithelial Cells. A.L. Rubin, N.L. Parenteau and R.H. Rice, J. Cell Physiol. 1989;138(1): 208-214.

- Reconstitution of Living Organ Equivalents from Specialized Cells and Matrix Biomolecules. E. Bell, M. Rosenberg, P. Kemp, N. Parenteau, H. Haimes, J. Chen, M. Swiderek, F. Kaplan, D. Kagan, V. Mason and L. Boucher. In Organes Artificiels Hybrides (Hybrid Artificial Organs), edited by C. Baquey and B. Dupuy. Colloque INSERM 1989 (177), pp. 13-28.

- The Living Skin Equivalent: Its Manufacture, Its Organotypic Properties and Its Response to Irritants. E. Bell, N. Parenteau, C. Nolte, P. Kemp, P. Bilbo, B. Ekstein, and E. Johnson, Toxic. In Vitro 1991; 56:591-596.

- Serial Cultivation of Normal Human Keratinocytes: A Defined System for Studying the Regulation of Growth and Differentiation. E. W. Johnson, S. F. Meunier, C. J. Roy, and N. L. Parenteau, In Vitro Cell. Devel. Biol. 1992; 28A(6):429-435.

- The Organotypic Culture of Human Skin Keratinocytes and Fibroblasts to Achieve Form and Function. N.L. Parenteau, P.R. Bilbo, C.J.M. Nolte, V.S. Mason, and M. Rosenberg. Cytotechnology, 1992;9:163-171.

- Skin in Complex Culture: The Transition from "Culture" Phenotype to Organotypic Phenotype. P.R. Bilbo, C.J.M. Nolte, M.A. Oleson, V.S. Mason, and N.L. Parenteau. J. Toxicol.-Cut. & Ocular Toxicol. 1993;12(2): 183-196.

- Development of a Stratum Corneum and Barrier Function in an Organotypic Skin Culture. C.J.M. Nolte, M.A. Oleson, P.R. Bilbo and N.L. Parenteau. Arch. Derm. Res. 1993; 285(8):466-474.

- Development of a Full-Thickness Living Skin Construct for Clinical Applications. Wilkins, L. M., Watson, S.R., Prosky, S.J. Meunier, S.F. and Parenteau, N. L. Biotechnology and Bioengineering 1994;43:747-756.

- Basement Membrane Assembly and Differentiation of Cultured Corneal Cells: Importance of Culture Environment and Endothelial Cell Interaction. Zieske, J.D., Mason, V.S., Wasson, M.E., Meunier, S.F., Nolte, C.J.M., Fukai, N., Olsen, B.R., and Parenteau, N.L. Exp. Cell Res. 1994; 214(2): 621-633.

- Cultured Skin as a "Smart Material" for Healing Wounds. Sabolinski, M.L., Mulder, G., Alvarez, O., Auletta, M. and Parenteau, N.L. Biomaterials 1996; 17(3):311-320

Papers

- In Vitro Skin Models. K. Kriwet and N. L. Parenteau. Cosmetics and Toiletries Magazine 1996;111:93-102.
- The Allogeneic Response to Cultured Human Skin Equivalent in the Humanized SCID Mouse. Briscoe, D.M., Dharnidharka, V.R., Isaacs, C., Downing, G., Prosky, S., Parenteau, N.L., and Hardin Young, J. Transplantation 1999 June 27;67(12):1590-9.
- Ultrastructural Arrangement of Melanosomes Donated to Keratinocytes within Chimeric Human Pigmented Living Skin Equivalent. Hardin Young, J., Prosky, S., Crews, J., Ross, R., Wilkins, L., Parenteau, N.L. Submitted.

Abstracts

- Specific Role of Calcium in Expression of Transglutaminase in Cultured Malignant Human Keratinocytes. 1984, A. L. Rubin, N. L. Parenteau and R. H. Rice, J. Cell Biol. 99:314a.
- Primate Involucrins: Sensitive Detection, Novel Solubility and Multiple Forms. 1985, N. L. Parenteau and R. H. Rice, J. Cell Biol. 101:374a.
- Effect of Glucose Levels on Collagen Degradation in a Living Skin Equivalent. 1988, V. S. Mason, H. B. Haimes and N. L. Parenteau, J. Cell Biol. 107(6):379a.
- The Living Skin Equivalent Model for Dermatotoxicity Testing. 1989, R. Gay, T. Class, M. Swiderek, H. Haimes, B. Pazzano, N. Parenteau and E. Bell. J. Cell Biol. 109:111a.
- The Organogenesis TESTSKIN_ Development and Validation Studies.1990, R. Gay, N. Parenteau, T. Class, M. Swiderek and E. Bell. In Vitro Cell Devel. Biol. 26:34a.
- Epidermal Migration Studied with a Living Skin Equivalent. 1990, N. Muthukumaran, P. Bilbo, G. Green, H. C. Hastings and N. Parenteau. J. Cell Bio. 111:441a.
- The Effects of TGF-beta on the Regulation of Differentiation in Normal Human Keratinocytes. 1990, N. Parenteau and H. C. Hastings. J. Cell Bio.111:347a.
- A Highly Differentiated Organotypic Skin Model for Dermatologic, Pharmacologic and Toxicologic Research. 1991, P. R. Bilbo, C. J. M. Nolte, C. Tighe, and N. L. Parenteau, J. Invest. Dermatol. 96:618a.
- A Dermal Model for In Vitro Wound Healing Studies. 1991, V. S. Mason, P. D. Kemp and N. L. Parenteau, J. Cell Biol, 115:114a.
- The Living Skin Equivalent as a Model for Studies of Barrier Function. 1992, C. J. M. Nolte, M. A. Oleson, P. R. Bilbo, R. J. Gay and N. L. Parenteau, J. Invest. Dermatol. 98:512.
- Effects of Serum Lipid Availability and Lipid Supplementation on the Stratum Corneum Fatty Acid Composition and Barrier Function of an Organotypic Skin Culture. 1992, M. A. Oleson, C. J. M. Nolte, P. R. Bilbo, and N. L. Parenteau. Molec. Biol. Cell 3 (suppl.):278a.

- Basement Membrane Assembly in Cultured Corneal Cells. J. D. Zieske, V. S. Mason, C. M. Nolte, Y. Muragaki, B. R. Olsen, and N. L. Parenteau. Presented as part of the "Hot Papers" section at the ASCB meetings, Denver, CO., Dec. 1982.

- Effect of Culture Environment and Cell-Cell Interaction on the Differentiation of Rabbit Corneal Epithelial Cells. V. S. Mason, J. D. Zieske, C. M. Nolte, Y Muragaki, B. R. Olsen and N. L. Parenteau, ARVO, May 1993.

Invited Talks

- How Well Does the Epidermis Differentiate *In Vitro*?, presented at the UCLA symposium on Tissue Engineering, Keystone, CO. 1990, N. Parenteau, P. Bilbo, C. Nolte and R. Gay.

- Organotypic Culture to Achieve Form and Function, presented at Cell Culture Engineering III, Palm Coast, FL, March 1992.

- Special Methods Workshop: Skin Cell Culture, New Techniques, Soc. Invest. Dermatol. Annual Meeting, May 1992.

- Methodologies and In Vitro Applications for Organotypic Skin Culture Models Symposium, New York, NY, June 1992.

- Use of Skin Equivalents in Development, presented as part of the UMDNJ continuing education program: "Insights into Evaluation of Topical and Transdermal Products." Nov. 1992.

- The Construction of Functional Models of Skin and Cornea using Cultured Cells and Collagen. N. L. Parenteau, V. S. Mason, C. M. Nolte, J. Zieske, Y. Muragaki, and B. R. Olsen. Presented at the American Chemical Society meetings, Symposium of Cell and Tissue Engineering, March 1993.

- Skin Equivalents: Achieving Biological and Functional Relevance. Oct. 1993, 6th International Symposium on Wound Healing and Wound Management, San Francisco, CA.

- Development of a Bilayered 'Skin Equivalent': from Basic Science to Clinical Use. A Keystone Symposium, Tissue Engineering, Feb. 1994, Taos, New Mexico.

- The Importance of Matrix and Tissue Structure for Cell Function and Persistence. XI Congress of the International Society for Artificial Cells, Blood Substitutes, and Immobilization Biotechnology, July 1994, Boston, MA.

- The Use of Cytokines and Related Strategies for Wound Healing, presented as part of the UMDNJ continuing education program: "Insights into Advances in Dermatological Therapies", Oct. 1994.

- The Use of a Cultured Skin Replacement for the Treatment of Difficult to Heal Wounds. Congress on Cellular Therapy and Tissue Engineering, BioEast'95, Washington, DC. (Member of the Organizing committee)

- In Vitro Effects of Human Keratinocytes on activated Peripheral Blood Lymphocytes (PBL). FASEB, Atlanta, GA., 1995.

- Establishing New Skin Tissue Using Engineered Skin Equivalents. Biochemical Engineering IX, Davos Switzerland, May 1995.

Invited Talks

- Cultured Skin Tissue for the Treatment of Chronic Ulcers. Gordon Conference on Epithelialization and Keratinization, July 1995.
- Development Experiences with Cultured Skin. Advances in Tissue Engineering, Rice University, School of Continuing Studies, Institute of Biosciences and Bioengineering, Houston Texas, August 1995.
- Utilizing Organotypic Skin Cultures: Benefits and Special Considerations, 4th Annual New Technologies Workshop, Microbiological Associates, Bethesda MD, September 1995.
- Cell Culture Engineering V, speaker and session co-chair, San Diego, CA, January 1995.
- The Design and Development of a Manufactured Cellular Product. Cell Culture Engineering V, Tissue Engineering and Somatic Cell Therapies, San Diego, CA., January 30, 1996.
- Georgetown University, Department of Cell Biology, Graduate Seminar Series, February 1996.
- The Use of a Bilayered Living Skin Construct for the Treatment of Difficult to Heal Wounds. Advances on Wound Healing, Burn Care and Infection Control, February 19-23, 1996.
- University of Minnesota, Bioengineering Program Seminar Series, April 1996.
- The Role of Cell-Cell, Cell-Matrix and Cell-Environment Interactions in Tissue Engineering. Biomedical Engineering Society: 1996 Annual Fall Meeting, October 3-6, 1996.
- Apligraf: Development of a Living Skin Equivalent. An Introduction to Graftskin-HSE (Human Skin Equivalent) (Apligraf) – A New Option for Treating Difficult-to-Heal Wounds, Hamilton Island, Australia, June 11, 1997.
- The Biology of Graftskin-HSE (Human Skin Equivalent) (Apligraf). World Congress of Dermatology satellite session: Apligraf – Redefining Wound Care, Sydney, Australia, June 15, 1997.
- The Role of Cell-Cell, Cell-Matrix and Cell-Environment Interactions in Tissue Engineering. The Art and Science of Wound Care, Orlando, FL., December 4-6, 1997.
- Living Skin Equivalents. Keystone Symposium (Wound Repair), Copper Mountain, CO, January 10-15, 1998.
- Tissue Engineering on Skin Substitutes. Georgia Institute of Technology, Atlanta, GA, April 1998.
- A Scientist's Perspective on Succeeding in Biotechnology, The Pittsburgh Tissue Engineering Initiative, Pittsburgh, PA, February 10-11, 1999.
- Tissue Engineering in Surgery, The Montreal General Hospital, McGill University, Montreal, Quebec, March 10, 1999.
- Organotypic Skin Construct for Clinical Use, Tissue Engineering and Cellular Culture, Congress Centre of Villa Gualino, Turin, Italy, May 29-30, 2000

Invited Talks

- De Novo Matrix Production and Regulation by Human Fibroblasts *In Vitro*. The First Symposium of the International Society of Matrix Biology, Philadelphia, PA, June 15-17, 2000.
- Apligraf: Design and Production of the Human Skin Equivalent, Satellite Symposium at the European Association for the Study of Diabetes, Jerusalem International Convention Centre, Jerusalem, Israel, September 17, 2000.
- "Cells" and "Implications: Industry Perspective", World Technology (WTEC) Workshop on Tissue Engineering, NIST Headquarters, Gaithersburg, MD, November 2-3, 2000.
- Human Skin Organotypical Cultures and Their Applications, 3rd Workshop on the Skin and It's Cells, Massachusetts General Hospital, Charlestown, MA 02129, November 13-17, 2000.
- Tissue Engineering: Interdisciplinary, Multi-disciplinary Technology, American Society of Mechanical Engineers, Tissue Engineering Symposium, Orlando, FL, November 5-10, 2000.

Figure 1. Ultrastructural analysis of Human Dermal Matrices by transmission electron microscopy (TEM). Banded collagen fibrils seen in serum and serum-free Human Dermal Matrices demonstrating the 67 nm periodicity characteristic of fibrils observed in neonatal foreskin and adult human skin.

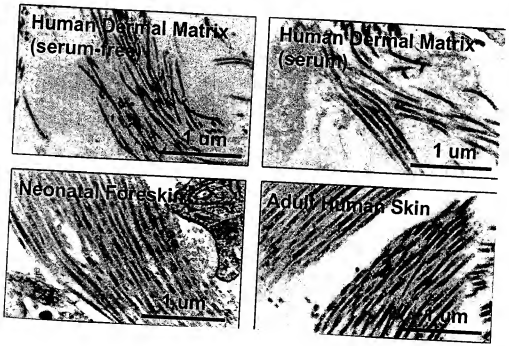

Table Decorin and biglycan content of Human Dermal Matrices. Decorin and biglycan contents were measured for an area corresponding to 44.2 cm^2. Results for decorin and biglycan content were obtained from an inhibition ELISA and are reported as ug core protein/construct ± SEM, n=3.

Construct	Decorin (ug)	Biglycan (ug)
Human Dermal Matrix (serum-free)	**308 ± 7.42**	**2.89 ± 0.11**
Human Dermal Matrix (serum)	**150 ± 25.1**	**1.90 ± 0.08**